# *In vitro* PROPAGATION OF *RORIDULA GORGONIAS* (RORIDULACEAE)

MICHAEL S. BODRI • Department of Biology • School of Science & Health Professions • North Georgia College & State University • Dahlonega, GA 30597 • USA • msbodri@ngcsu.edu
MATTHEW R. OPEL • Department of Ecology and Evolutionary Biology • University of Connecticut • U-3043 • Storrs, CT 06269 • USA

Keywords: conservation: *Roridula*—cultivation: tissue culture, *Roridula gorgonias*.

Two species of proto-carnivorous woody shrubs are represented in the genus *Roridula* (Roridulaceae Martinov, *nom. cons.*): *R. dentata* and *R. gorgonias* (Conran 2004). They are the only members of the Roridulaceae. These plants are endemic to the fynbos of the South African Cape Floristic Region, an area subject to periodic fires and marked by poor impoverished soils, warm, dry summers and comparatively cool, rainy winters (Cowling & Richardson 1995). Both species produce a sticky resin from glandular tentacles on their lanceolate leaves which entrap insects. Specialized hemipteran bugs feed upon the trapped insects and nutrients from bug feces are absorbed by the plants' leaves (Ellis & Midgley 1996) which show specialized anatomical features that may assist nutrient absorbtion (Anderson 2005). Analysis of $\delta^{15}N$ levels in leaves (Midgley & Stock 1998) suggests that *Roridula* plants obtain much of their nitrogen requirements from insect prey and probably meet the criteria for true carnivory (Givnish 1989).

The interim 2007 South African IUCN Red Data list does not list *Roridula dentata* while *R. gorgonias* is listed as a least concern (LC) species. Even though *R. dentata* has had a historically wide distribution, frequent fires, wrong season fires, and habitat destruction by agriculture have eliminated populations previously documented by herbarium collections. It is possible that *R. dentata* may be given vulnerable (VU) or endangered (EN) status based upon current data (Royal Botanic Gardens 2007). The LC designation for *R. gorgonias* may change due to the same criteria as indicated for *R. dentata*.

Because seed of *R. dentata* may be produced in quantities too small for seed banking or to regenerate the soil seed bank *in situ*, this study of *R. gorgonias* was undertaken to determine *in vitro* propagation techniques that could be applied to *R. dentata* should the need arise for an alternative means to preserve germplasm. There is considerable horticultural interest in *Roridula* among botanical gardens and carnivorous plant hobbyists but because propagation by seed or cuttings is slow and difficult, plants are rarely if ever available in the horticultural trade (Opel 2005). *In vitro* methods could also be used to propagate plant material for horticultural and scientific use. Results presented in this paper describe the successful axenic propagation of *R. gorgonias* from seed.

Seeds were obtained from cultivated plants at the University of Connecticut Ecology and Evolutionary Biology Plant Growth Facility (voucher at CONN, #131684, M. R. Opel 261) and randomly divided into three groups: no scarification (n=13); scarification prior to surface sterilization (n=10); and scarification following surface sterilization (n=11). Scarification consisted of removal of a portion of the seed coat with a sterile scalpel blade.

Surface sterilization was achieved by 10 min exposure to a 10% (v/v) bleach solution. Seeds were then transferred in groups of 3-4 to 10 ml modified Murashige and Skoog (MS) media (Murashige & Skoog 1962) without rinsing. Basal MS medium consisted of micronutrients and iron used at full strength but macronutrients diluted to G strength (hereinafter referred to as G MS). Sucrose (20 g $l^{-1}$), MS vitamins (106 mg $l^{-1}$, Sigma-Aldrich Co., St. Louis, MO, USA), phytagel™ (6 g $l^{-1}$, Sigma-Aldrich Co., St. Louis, MO, USA) at pH 5.8 were added prior to autoclaving. Cultures were maintained at 25°C under cool-white fluorescent tubes with a 14:8-h photoperiod.

Figure 1: *Roridula gorgonias in vitro*. Photograph by Michael Bodri.

Figure 2: *Roridula gorgonias* root system *in vitro*. Photograph by Michael Bodri.

Seedlings were allowed to reach 5-6 cm length prior to subculturing onto G MS media containing 2 mg $l^{-1}$ kinetin and 0.2 mg $l^{-1}$ of the potassium salt of indole-3-butyric acid (K-IBA). Following stem proliferation and root production, plantlets were subcultured back to ? MS prior to greenhouse establishment.

Seeds, regardless of scarification regimen, began germinating within 23 d. Approximately 60% of all seed germinated (7/13 no scarification, 7/10 scarified prior to sterilization, 6/11 scarified after sterilization) with no significant difference between treatments (Fisher exact test, Fisher 1958). Root proliferation occurred prior to shoot proliferation, approximately one month post subculture, with adventitious roots developing from the stems of the seedlings above the culture media as well as within the media. Shoot proliferation occurred shortly thereafter from the base of the plants at the level of media rather than from the axillary buds not in contact with the media. After subculture in G MS supplemented with 2 mg $l^{-1}$ kinetin and 0.2 mg $l^{-1}$ K-IBA for 10-14 wks, the young shoots were grown enough to separate into individual plantlets. Separated plantlets developed normally when subcultured onto G MS without auxin and cytokinen (see Figures 1, 2). Plantlets readily established in the greenhouse after removal from culture media, the roots washed in tap-water and the plantlets transferred to plastic pots containing a non-sterile well-draining mixture of peat, sand and expanded clay material. Plants were covered with glass domes to increase humidity during a 4-wk acclimation period. We are still refining and improving this technique. For example, a current challenge is in long-term establishment of mature plants outside of the sterile conditions. For as yet unknown reasons, some plants that are potted and grown in greenhouse conditions (that support conventional, seed-grown *R. gorgonias*) may thrive for many months, then decline and die. This may be more of an issue related to their exacting cultivation requirements instead of their *in vitro* origins.

These results indicate that seed scarification and rinsing of seed with sterile water following surface sterilization is not necessary for successful germination of *R. gorgonias*. MS media used at G strength macronutrients and 20 g $l^{-1}$ sucrose is a suitable media that supports axenic growth of this species and, if supplemented with kinetin and K-IBA, will initiate shoot and root proliferation.

The method described allows for the successful axenic production of *R. gorgonias*, and is likely applicable to *R. dentata*, as well.

References


Anderson, B. 2005. Adaptations to foliar absorption of faeces: a pathway in plant carnivory. Annals of Botany 95: 757-761.

Conran, J.G. 2004. Roridulaceae. In: Kubitzki K (ed) The families and genera of vascular plants. Vol VI. Flowering Plants. Dicotyledons. Springer, New York.

Cowling, R., and Richardson, D. 1995. Fynbos: South Africa's Unique Floral Kingdom. Fernwood Press, Vlaeberg, South Africa.

Ellis, A.G., and Midgley, J.J. 1996. A new plant-animal mutualism involving a plant with sticky leaves and a resident hemipteran. Oecologia 106: 478-481.

Fisher, R.A. 1958. Statistical methods for research workers. 13th ed. Hafner, New York.

Givnish, T.J. 1989. Ecology and evolution of carnivorous plants. In: Abrahamson WG (ed) Plant-animal interactions. McGraw-Hill, New York.

Midgley, J.J., and Stock, W.D. 1998. Natural abundance of ? $^{15}N$ confirms insectivorous habit of *Roridula gorgonias*, despite it having no proteolytic enzymes. Annals of Botany 82: 387-388.

Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco cultures. Physiologia Plantarum 1: 474-497.

Opel, M.R. 2005. *Roridula*, a carnivorous shrub from South Africa. Carniv. Pl. Newslett. 34: 106-110.

Royal Botanic Gardens 2007 (citation date). Kew Millennium Seed Bank Project